# Effect of *Laurus nobilis* extract on different cancer cell lines

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## Abstract:

Bay leaf belongs to the family Lauraceae, and it is one of the most popular culinary spices in all Wstern counteries. Bay leaf has been used as herbal medicine and it has pharmacological activity, antibacterial, antifungal, antidiabetes, antiinflammatory effects and cytotoxic activities. In this stud, the effect of *laurus nobilis* hexan extract on three different cell lines was investigate in an attempt to identfy its potential activity. Cell viability and proliferation were assessed against different concentrations of *laurus nobilis* hexan extract (bay leaf) and reveled a remarkable inhibition of $AMN_3$, REF and Hela cell lines in a dose dependent manner. Activity against $AMN_3$ (mouse mammary adenocarcinoma), REF (rat emberyo fibroblast) and Hela (cervical human carcinoma cell line taken from Henrietta Lacks established 1951) was established with OD of 0.054, 0.05 and 0.1 respectively at the concentration 500 µg/ml with incubation period 24 h. The inhibition rate was 80%, 77% and 69 % for $AMN_3$, REF and Hela respectively.

## Introduction:

Human epidemiology and animal studies have indicated that cancer risk may be modified by dietary components. The naturally occuring, non-nutritive chemical components of fruits, vegetables, grains, nuts, tea and seeds, which are commonly referred as phytochemicals or active compounds, may prevent or reduce the risk of cancer[(1)].

***Laurus nobilis*** (Lauraceae), the laurel or bay laurel, is an evergreen tree widespread in the Mediterranean area and Eueope, and as a folk medicine, the decoction or tea of bay leaves is often used as a carminative, intestinal and gastric antispasmodic, against diarrhea, for rheumatic pains, in diseases of the respiratory tract, as a cough sedative, to treat asthma and cardiac diseases [(2-3)]. Previous phytochemical investigations have led to isolation of several classes of secondary metabolites of laurel leaves, particually sesquiterpene lactons [(4-5)], alkaloid [(5)], monoterpene and germacrane alcohols [(6-7)], flavonoids [(8)] and glycosides [(9)]. Bay leaves are commonly used as a spicy, aromatic flavoring for soaps, fish, meats, stews, puddings, vinegars, and beverages. The essential oil is used by the cosematic industry in creams, perfumes, and soaps [(10)]. Many of the

important compounds have been shown to possess various pharmacological effects, with antimicrobial [(11)], immunomodulating [(12)], and cytotoxic activities [(13)]. Some derivatives isolated from laurel leaves shown to be able to induce apoptotic chromatin condensation in leukemia cells (HL-60) [(14)], indicating their possibility as leads in the development of new classes of antileukemic drugs. For this reason, this plant is concedered in our ongoing studies on antiproliferative agents from vegetable sources [(15)].

Cell death can follow distinct pathways, apoptosis or necrosis. Necrosis appears to be the result of acute cellular dysfunction in response to sever stress condition or after exposure to toxic agents [(15)]. Apoptosis is a physiological form of cell death that occurs during development of multicellular organisms or during the development of the immune response. In addition, the apoptotic program can be activated in response to stress conditions, toxins chemicals, phsical agents and others [(16)].

The aim of this study was designed to the investigate the possible beneficial effects of *laurus nobilis* hexan extract (bay leaf) on $AMN_3$, REF and Hela cell lines in an attempt to identif its cytotoxic activit.

## Material and Methods:

- Plant material and preparation of the extract:

Dried leaves of laurel were collected locally for aqueous extract the leaves were ground using an electric blander, about 10 g of the powder plant were soaked in 100 ml distilled water and putted on a mixer at 30 ºc for 6 hrs. The mixture was then filtered through a filter paper to remove all the residual materials.The extract was then filtered using 0.2 mm wattman filter papers and different concentration made and then kept at 4ºC until used. Then, it dried at 45˚C by using hot air oven, with circulatory fan, and kept at 4˚C until the use.

- Cytotoxicity test using ELIZA assay:

For this test, the extract were weighed (0.05 g) and dissolved in phosphate buffer saline (PBS) and dimethylsulphoxide (DMSO) to prepare extract solution at 1000 µg/ml. The following dilutions of extract were then prepared: 500, 400, 300, 200 and 100 µg/ml and kept at 4˚C until the use. .

- Cell line and culture:

This *in vitro* method was used to establish the effect of the hexan extract of *Laurus nobilis* on (Hela, AMN-3 and REF cell lines). Solutions were prepared according to ICCMGR standard method. These cells were maintained in RPMI – $_{1640}$ media with 10% (v/v) bovine serum and incubated at 37˚C in a humidified atmosphere containing 5% $CO_2$ and 95% air.

- Cytotoxic assay:

The cytotoxic assay was measured using the crystal violate staine [(17)]. In brief, the extract re dissolved in DMSO then diluted by serum free media (SFM) and (500, 400, 300, 200 and 100 µg/ml) concentrations perpared, then tumor cells were seeded in 96- well microplate. After 24 h incubation at 37˚C, the old

media was changed with new media SFM containing the serial concentrations of each extract, and then plate incubated for 24 hr of all cell lines and humidified incubator at 37˚C containing 5% $CO_2$. After finishing the exposure periods, the old media was replaced with 200 µl/well of crystal violate dye and incubated the plate 20 min at 37˚C, the wells were washed with warmed PBS. The plates leaved for 15 min at room temperature and then the absorbancy of each well was readed by ELISA reader at 492 nm.

## Statistical analysis:

All data obtained based on statistical analysis by SPSS and the results were considered statistically significant with ($P<0.05$).

## Results:

The effect of *laurus nobilis* bay leaf hexane extract on three types of cancer cell line is shown in figure (1). The figure shows that the extract have different cytotoxic activity on the various cell lines tested.

The results indicated increasing in cytotoxic effect of the extract concentration on the same cancer cell line and between the same extract.

REF and $AMN_3$ was most sensitive cell line to bay extract while Hela was the less sensitive cell line to the same extract concentrations, this depends on the inhibitory effect of each concentration on each cancer cell line. The inhibition rate of REF and $AMN_3$ was 80 % and 77 % respectively, while the inhibition rate of the extract concentration at 500 µg/ml gives 69%. There is no significant difference in the inhibition rates of the extract concentrations at the level ($p\leq0.05$).

Recent studies, including ours, have shown that *laurus nobilis* bay leaf hexane extract cytotoxic activity against A2780 ovarian cancer cell line with inhibition rate 98% [(18)].

In other studies showed the cytotoxic effect of essential oils and other identified constituents from different plants including *laurus nobilis* against *in vitro* human cancer cell lines [(19)].

Different extracts from *laurus nobilis* using n-hexan, ethanol and water were done and evaluated for cytotoxic properties using the Brine Shrimp bioassay. Only n-hexan exhibited cytotoxic activity [(20)].

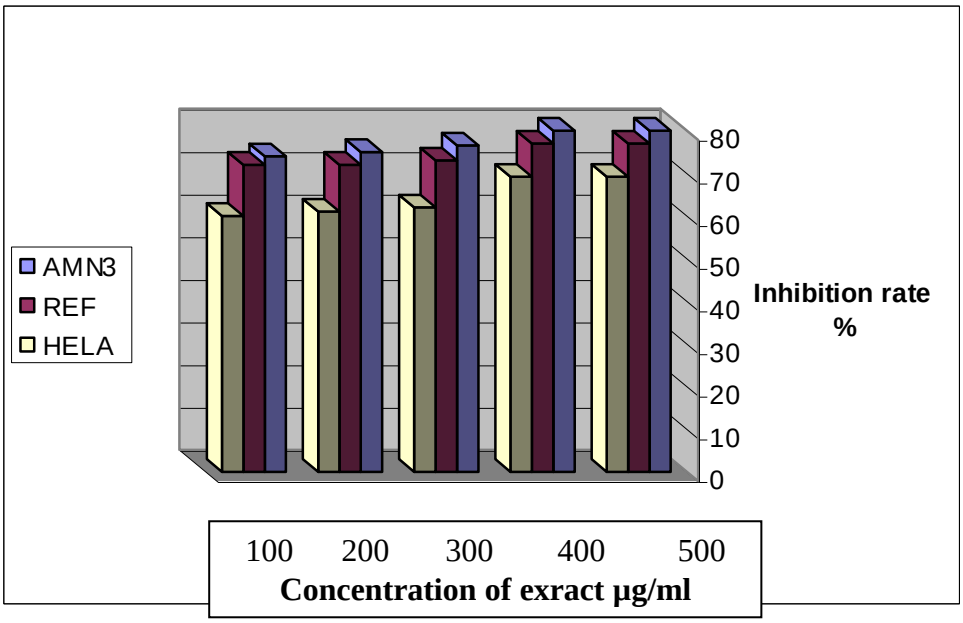


fig 1: In *vitro* cytotoxic effect of *laurus nobilis* bay leaf hexan extract on different cancer cell lines.

**Discussion:**

Plant derived extracts have been historically considered as important alternative remedies for enhancing immune status and prevention and treatment of chronic diseases [21].

In this study, hexan extract (supposed to contain mainly polar compounds) were used. The watery extract were also done in this study and gives no inhibition to cancer cell line and minimum than that appeared in hexan extract, so the result of water extract were not shown in the study.

Since it is known that different cell lines might exhibit different sensitivities towards a cytotoxic compound, the use of more than one cell line is therefore considered necessary in the detection of cytotoxic compounds. Bearing this in mind, three cell lines of different histological origin were used in the present study. As can be seen from figure (1), cell type cytotoxic specificity is observed in *laurus nobilis* bay leaf hexan extract.

Cell type cytotoxic specificity of plant extracts is likely to be due to the presence of different classes of compounds in the extract. The results of the present study indicate the presence of cytotoxic activity *laurus nobilis* bay leaf hexan extract at inhibition rate (80%, 77% and 69 %) for $AMN_3$, REF and Hela respectively.

Several sesquiterpene lacones isolated from the leaves of *laurus nobilis* were tested *in vitro* against three human umor cell lines Jurkat (T lymphoblastoid leukaemia), HL-60 (promylocytic leukaemia) and LoVo (intestinal adenocarcinoma) and showed an inhibition toward these cells [22].

Apoptosis is an active, energy-dependent mechanism in which cells participate in their own destruction wheras necrosis is a passive process of cellular metabolic collapse followed by cellular disintegration [23].

In conclusion, *laurus nobilis* hexan extract has shown a cytotoxic effect on $AMN_3$, REF and Hela cell lines. These results suggest that bay leaf can be a promising anti-cancer therapeutic agents for several cancer cells.

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# تاثير مستخلص ورق الغار على مختلف الخلايا السرطانية

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الخلاصة

ورق الغار يعود الى العائلة الغارية، وهي من اشهر التوابل المستخدمة في كل بلدان الغرب. ورق الغار استعمل كدواء عشبي وله فعالية صيدلانية ضد البكتريا والفطريات وضد السكري ، وضد الالتهابات وفعالية سمية للخلايا.

في هذه الدراسة تم دراسة تاثير المستخلص الهكساني لورق الغار ضد الخلايا السرطانية الثلاث ($AMN_3$, REF and Hela) لمعرفة تاثير عليها.

تم معرفة حيوية الخلايا باستخدام تراكيز مختلفة من المستخلص الهكساني لورق الغار واظهر قابليته الواضحة على تثبيط نمو الخلايا الثلاث اعتمادا على تركيز المستخلص المستخدم، تم قراءة ذلك بواسطة مقياس الطيف الضوئي 452 OD.

افضل تركيز كان 500µg/ml والذي اعطى افضل تثبيط لنمو الخلايا ($AMN_3$, REF and Hela) خلال ال 24ساعة الاولى من التعريض للمستخلص وكان التثبيط % 69 and 77% ,80% على التوالي.